

## Amendment and Response

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Serial No.: 09/180,340

Confirmation No.: 6674

Filed: 20 August 1999

For: STABLE RECOMBINANT YEASTS FOR FERMENTING XYLOSE TO ETHANOL

C2  
Amended

(a) transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a first selection marker, and

(b) repeatedly replicating the cells from step (a) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, promoting the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

C3

18. A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a selection marker, the exogenous DNA being flanked on each end by a DNA sequence homologous to a reiterated sequence of DNA of the host;

(ii) repeatedly replicating the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative plasmid in subsequent generations of the progeny cells and result in progeny cells each containing multiple integrated copies of the exogenous DNA; and

(iii) replicating the progeny cells from step (ii) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

C4

23. A yeast which ferments xylose to ethanol, comprising:

multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and

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04  
Conclude

xylulokinase fused to non-glucose inhibited promoters, the yeast fermenting glucose and xylose simultaneously to ethanol and substantially retaining its capacity for fermenting xylose to ethanol for at least 20 generations when cultured under non-selective conditions.

05

25. A yeast which ferments xylose to ethanol, comprising:

multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, the yeast fermenting xylose to ethanol and substantially retaining its capacity for fermenting xylose to ethanol when cultured under non-selective conditions for at least 20 generations.

06

27. A method for fermenting xylose to ethanol, comprising fermenting a xylose-containing medium with a yeast of claim 1, 22, 23, 24, 25, or 26, to produce ethanol.

*eukaryotic, yeast or bacteria*

07

30. A method for producing cells having multiple integrated copies of an exogenous DNA fragment, comprising:

replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence and containing the exogenous DNA to produce multiple generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.